ELECTRO-OPTICAL MECHANICAL INSTRUMENT

BACKGROUND OF THE INVENTION

The present invention relates to optical scanners, and more particularly to a quasi con-focal microscope scanner in which the specimen and the scanner are simultaneously moved relative to each other.

Micro array biochips are currently being developed by several biotechnology companies. Micro array biochips are small substrates containing thousands of DNA sequences that represent the genetic codes of a variety of living organisms including human, plant, animal, and pathogens. They provide researchers with volumes of information in a more efficient format. Experiments can be conducted with significantly higher throughput than previous technologies offered. Biochip technology is used for genetic expression, DNA sequencing of genes, food and water testing for harmful pathogens, and diagnostic screening. Biochips may be used in pharmacogenomics and proteomics research aimed at high throughput screening for drug discovery. High-speed automated biochemistry may lead to drugs for treating illnesses including HIV, cancer, heart disease and others.

DNA sequences are extracted from a sample and are tagged with a fluorescent probe, a molecule that, when "excited" by a laser, will emit light of various colors. These fluorescently tagged DNA sequences are then spread over the chip. A DNA sequence will bind to its complementary (cDNA) sequence at a given array location. A typical biochip contains a two-dimensional array of thousands of cDNA sequences, each one unique to a specific gene. These cDNA sequences may be "printed" on the chip in several ways. Once the biochip is printed, it represents thousands of experiments in an area usually smaller than a postage stamp.

The chip is then ready to be scanned and analyzed with a scanning laser microscope using a dichromic beam splitter. However. The dichromic beam splitter has two drawbacks. Each time a specimen with a different dye is to be read, the beam splitter must be changed to match the different wavelengths

of operation of the new dye and the number of multiple dyes that can be simultaneously interrogated is usually limited to two.

The microscope collects data from successive "pixels" which are best dimensioned in microns. There are essentially two types of optical scanner, namely scanners that move scan heads and associated optics over stationary specimens, and scanners that move the specimens relative to stationary optics. Known scanning microscopes must therefore precisely align the optics of a moving scan head with the beam of a stationary laser, or alternatively carry the laser on the moving scan. A stationary laser can be aligned with a moving scan head only at relatively slow speeds, and therefore the scan speed of the system is inherently limited. The alternate system requires a relatively large scan head to carry the associated optics whereby the relatively great size and weight also effectively limits the scan speed.

SUMMARY OF THE INVENTION

The present invention provides a scanning laser microscope which can be used to scan biochips and display the information embodied in the fluorescent energy emitted by the individual dots as a pictorial representation of the array on a T.V. monitor. The means of interrogation is laser light (the excitation energy). The laser light excites the fluorescein that is contained in the fluorescent dyes. The fluorophores will subsequently emit light of a wavelength that is longer than the wavelength of the excitation energy. Thus, by using a beam splitting mirror, the number of different dyes that can be interrogated simultaneously is unlimited.

The optical diagram is a quasi con-focal microscope, *i.e.*, only an area the size of approximately one pixel is illuminated (excited) and observed (detected) at a time, however the size of the illuminating spot is not nearly as closely matched to that of the detected spot as it is in a pure con-focal microscope, in fact the former is about 10X larger in diameter than the latter.

The emitted light is conducted by lenses, mirrors, and optical filters to a detector, where it is converted into computer readable data.

The horizontal, or line scan (the X scan) is mechanized by moving the objective lens of the system rapidly back and forth in the X direction across the shorter length of a microscope slide specimen collecting data in each direction. The slide specimen does not move in the X direction as the vertical, or page scan (the Y scan) is mechanized by moving the slide specimen in the Y direction, incrementally advancing the slide each time the X scan is about to start a line.

The information is preferably processed so that it may be displayed in a convenient format such as tables, histograms and the like. The pictorial or image-processed information can thereafter be stored on a hard drive and sent to a hard copy printer, transmitted to a LAN, or transmitted over the Internet.

BRIEF DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is a detailed perspective view of an optical instrument of the present invention;

Figure 2 is a plan view of a slide specimen of the present invention showing the movement of the scanning objective lens;

Figure 3 is a side view of the first drive mechanism; and

Figure 4 is a top view of the second drive mechanism.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

An optical instrument 10 of the present invention is generally shown in Figure 1. As will be further described below the optical instrument 10

generally includes a transmitter 12 that emits an optical signal 14, a beam splitting mirror 20 having an opening 22, a reflector assembly 30 which directs the optical signal 14 onto a specimen 90, a detector assembly 40 which detects a reflected optical signal 44 from the specimen 90, a first drive mechanism 50 for varying the position of the optical signal 14 on the specimen 90, and a second drive mechanism 66 for varying the position of the specimen 90 relative to the optical signal 14.

Figure 1 illustrates the main components of the optical instrument 10 and the optical signal 14 path. The means of interrogation is preferably laser light and more than one laser can be incorporated into the system. Further, various types of lasers may be employed, such as argon-ion, semiconductor diode, and other similar solid state lasers. In the preferred embodiment, a plurality of lasers 12A-C, each operating on a different wavelength, are shown.

The optical signals 14A-C are each first transmitted through a beam correcting lens 16A-C and then through a continuously variable neutral density filter 18A-C, which is employed to adjust the intensity of the beam. The variable neutral density filter 18A-C can be an addressable array of several fixed neutral density filters of different densities, a pair of polarizers of which one is rotatable, or a rotating polarization retarder, in front of a polarizer.

To direct the optical signal 14, the reflector assembly 30 includes a plurality of turn mirrors 32A-C. Each optical signal 14A-C is folded as appropriate by the turn mirrors 32A-C to a beam combiner 34A-C. The beam combiner is preferably a know dichroic filter which transmits light of one wavelength while blocking others. The individual optical signal are thereby collected into a combined beam along a first path which then passes through the opening 22 in the beam splitting mirror 20. The combined beam is then directed to a 90 degree fold mirror 36 located immediately above the scanning

objective lens 52. The fold mirror 36 reflects the combined optical signal 14 into the scanning objective lens 52, which in turn is focused onto the specimen 90, thus creating a scanning illumination spot. The embodiment shown in Figure 1 shows three laser transmitters 12A-C, however, those skilled in the art will realize that additional lasers can be used to interrogate multiple dyes of different fluorescent properties in the specimen 90 simultaneously or sequentially. The optical signal from each additional laser is located downstream from the last laser and is brought onto the system optical axis *via* reflection off a dichromic beam combiner.

When illuminated by the combined beam the fluorophores will emit energy all around, *i.e.*, into 4 pi directions. It is imperative to collect as much of this energy as possible, so it is preferable to employ a custom designed objective lens 52 such as one, for example only, with an NA=0.9, air-coupled 4. The objective lens 52 preferably outputs a beam of emitted energy concentric with the laser beam, having a diameter about 10X larger than that of the laser beam.

After reflecting from the specimen the fold mirror 36 located above the scanning lens 52 will fold the reflected optical signal 44 along a second path. The reflected optical signal 44 is again directed by 90 degrees towards the beam splitting mirror 20. The latter will fold the emission beam 90 degrees away from the combined optical signal first path, except for a very small central portion in the middle as determined by the opening 22 in the beam splitting mirror 20. It can be seen that a for a portion of the path the original combined optical signal 14 traveling along the first path, and the reflected optical signal 44 traveling along the second path, have a common path segment. This common path segment is shown between the beam splitting mirror 20 the fold mirror 36, and the scanning lens 52.

The reflected optical signal 44 reflecting from the opposite side of the beam splitting mirror 20 will then pass through a plurality of beam splitters

38A-B to separate the combined signal into individual signals 44A-C. Each individual signal 44A-C passes through an emission filter 46A-C, and will then be focused by a detector lens 48A-C into a pinhole. The pinhole acts as the field stop of the system, *i.e.*, it defines the size of the scanning detection aperture on the slide. Finally, the individual signals 46A-C diverts once through the pinhole until it impinges onto a detector 42A-C.

As shown in Figure 2 the horizontal, or line scan (the X scan) is mechanized by moving the objective lens 52 of the system rapidly (20 Hz or so) back and forth in the X direction across the shorter length of a microscope slide specimen 90 (commonly 1 inch wide), collecting data in each direction. Note that the slide specimen 90 does not move in the X direction. The vertical or page scan (the Y scan), is mechanized by moving the slide specimen 90 in the Y direction, incrementally advancing the slide specimen 90 each time the X scan is about to start a line scan as further described below.

Figure 3 shows the first drive mechanism 50 for varying the position of the combined optical signal on the specimen 90. The first or X scan mechanism preferably employs a galvanometric torque motor 54 to rotate a sector-shaped cam 56 over an angle between +40 degrees, and -40 degrees. The circular portion of the cam 56 is connected to the carriage 58 *via* a set of roll-up, roll-off thin, high-strength steel wires 66A-B. The scanning objective lens 52 is attached to the carriage 54. The radius of the cam 56 is such that its degree rotation will cause the carriage 58 to travel a linear distance along a rail 60 commensurate with the length of the X scan pattern of the objective lens 52.

Figure 4 shows the second drive mechanism 70. The second or Y scan mechanism employs a stepper motor 72 to drive a precision screw 74 in a known manner. The nut 76 on the screw 74 is attached to the carriage 58, so that any rotation of the screw 74 will cause the carriage 58 to move along a linear rail 60. The carriage 58 in turn is equipped with a tray 76. The tray

76 is equipped with appropriate retainers 78 to hold a specimen slide 90 in a position and orientation which is repeatable within an accuracy required by optical focus and alignment criteria. The rail of the linear slide and the stepper motor 72 are attached to the frame of the Y scan mechanism.

In an alternate embodiment, the carriage 58 is pivotally mounted such that the carriage 58, and thus the objective lens 52, move in an arcuate motion. The arcuate motion is thereafter converted into linear motion by know computer mapping programs.

The frame of the Y scan mechanism is further attached to the carriage of a vertically oriented linear slide. The rail of the slide is mounted to the main frame of the reader system. The carriage is supported by a precision screw, the nut of which is attached to the frame. The screw is turned causing the Y scan mechanism, and with it the slide holding the specimen, to move toward or away from the objective lens, thus affecting a focusing sequence.

The foregoing description is exemplary rather than limiting in nature. Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims the invention may be practiced otherwise than as specifically described.